Characterization and Description of Heat-tolerant *Bacillus* species Isolated from Spacecraft Assembly Facility

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Forward contamination of samples with cells or biomarkers from Earth would seriously compromise interpretation of results of a Space sample return mission. In ongoing investigations to map and archive the microbial footprints in various components of spacecraft and its accessories, we have examined the microbial populations of the Jet Propulsion Laboratory, Spacecraft Assembly Facility (SAF). We have exposed witness plates that are made up of spacecraft materials and or painted with spacecraft qualified paints for ~7 to 9 months. In the initial studies reported here, we have examined the total cultivable aerobic heterotrophs, and heat-tolerant (80°C for 15-min.) spore-formers. The results showed that the witness plates coated with spacecraft qualified paints attracted more dust particles than the non-coated stainless steel witness plates. Among four paints tested, witness plates coated with NS43G (an off-white conductive paint [silicate binder]) accumulated the highest number of particles, hence attracted more cultivable microbes and spore-formers. The microbiological examination revealed that the SAF High Bay-1 harbors mainly Gram-positive microbes and mostly spore-forming *Bacillus* species. Most of the isolated microbes were heat resistant to 80°C and grew well at 60°C. Based on the morphology, and physiology, 28 isolates were chosen for further study. The phylogenetic relationships among these heat-tolerant microbes were examined using a battery of morphological, physiological, molecular and chemotaxonomic characterizations. Sequence analysis of nearly complete sequences of 16S ribosomal RNA revealed that most of the microbes in SAF are *Bacillus licheniformis*. By 16S rRNA analysis, the isolates fell into seven clades: *Bacillus licheniformis, B. pumilus, B. cereus, B. circulans, Staphylococcus epidermidis, Planococcus* sp. and *Micrococcus lylae*. Based on DNA-DNA hybridization studies we could conclude that 3 of the 19 *Bacillus* strains tested are novel bacterial species. Likewise, one of the five Gram-positive coccoid-shaped bacteria needs new description. In addition to the *Bacillus* species, the isolation of human-associated microbes such as *S. epidermidis* indicates secondary contamination by human activity in SAF.

The search for life on Mars in the 2008 sample-return mission will likely involve ultra-sensitive detection of fossil biomarkers. Forward contamination of cores with cells or biomarkers from Earth would seriously compromise interpretation, particularly since meteorite exchange between Earth and Mars indicates a finite probability that Martian life could resemble life on Earth. Forward planetary protection requires unprecedented control of the bioburden carried from Earth to Mars particularly in the area of the sample cache.

Microorganisms in spacecraft assembly facility (SAF) may influence quality of spacecraft. Microbes of concern in SAF are those that are commonly found in its territory (habitat-associated), those that indicate the sanitary or quality of hygienic practices (human-associated), and those that under certain conditions could cause human illness. Primary sources of microbial contamination are likely to include soil and dust, air, earth and sources that are very difficult to control. The secondary source includes air, handlers, cross-contamination, equipment and buildings. Secondary sources of contamination are controlled by good manufacturing practices. The microbes of concern in SAF are primarily spore-forming bacteria and microbes that are indigenous to human skin, hair, and respiratory tract (Puleo et al., 1977). Standard industry practices control human-associated microbes. Bacterial spores, particularly those in the *Bacillus* genus, are common in spacecraft industry. Bacteria do not normally replicate in these highly desiccated, low-nutrient environments and as such high numbers of vegetative bacteria could indicate recent contamination from a secondary source. Typically, SAF can be expected to contain low numbers and a limited variety of microbes (Puelo et al., 1975). More information on the source and control of microbes in SAF is needed to answer the concerns currently facing the industry.

In the past few years, due to the use of molecular methods, our knowledge of microbial diversity has increased dramatically, not only from a phylogenetic and taxonomic perspective but also from an ecological basis. We now know that microorganisms exist in every conceivable place on Earth, even in extreme environments. Temperature may be the only limitation as to where they can and cannot exist and/or function. As more small subunit rRNA sequence information becomes available there is a real need to start turning the information into knowledge that can be applied to better elucidate and understand structure-function relationships within ecosystems, develop new culturing methods, and discover new products and processes.

In an ongoing investigations to map and archive the microbial footprints in various component of spacecraft and its accessories, we have examined the microbial populations of a SAF High Bay #1 at Jet Propulsion Laboratory (hereafter called as SAF). Witness plates from SAF were analyzed for contaminant composition and viability. In order to determine the taxonomic position of the strains that had been isolated from SAF and could not be identified accurately by phenotypic characterization, their 16S rRNA was sequenced. Further to support these data, FAME analysis and DNA-DNA hybridization were carried out. It may not be enough to know who is out there. One needs to assess the metabolic plasticity and regulation of the microbial community in order to better understand how the community functions under different conditions. The microbial population or diversity is an integral part of the ecosystem's function rather than a result of reacting to or upon ecosystem function. Since the microflora of SAF have direct impact on the quality of the product, the microbial diversity of this facility would give some insight and knowledge to eradicate these microorganisms and improve the quality of the spacecraft being built.

MATERIALS AND METHODS

Spacecraft assembly facility. The dimension of the SAF 1 is 80' wide, 120' long, and 44' 4" high. Relative humidity was controlled at 40 ± 5% with a cap at 45% and the average temperature was maintained at 20 ± 5°C. Personnel entry into this controlled facility was minimal and carefully monitored by setting a series of rigorous procedures. For example, people must clean their shoes in a provided mechanized shoe-cleaner, followed by taking an air shower before entering the ante-room. The ante-room provides all clean room garments including shoes, booties, gowns, caps etc. This SAF room was controlled by qualified contamination control people with a periodic check to maintain a Class 100,000 clean room level.

Collection of dust particles on witness plates. Witness plates of a specified size (1"x2") were used. Stainless steel plates were ultrasonically cleaned in acetone (5 to 10-min) followed by iso-propanol (5 to 10-min). After air drying, the plates were then sterilized either by heat at 175°C for 2 hours (Ref.) or by hydrogen peroxide gas plasma sterilization using Johnson and Johnson STERRAD® 100 System (Ref.). Four commonly used spacecraft qualified paints, coated on aluminum witness plates were also prepared (per JPL Process Specifications) and followed by sterilization in hydrogen peroxide gas plasma. The pre-sterilized witness plates made of stainless steel and four different paint-coated aluminum were exposed in a JPL spacecraft assembly facility. Stands that carry these witness plates were about 6 feet high to minimize contamination from human exhalation or sweat, thus allowing collection of dust particles that were naturally falling out on the witness plates in the assembly area. Exposure time, specifications of paints and positions of the witness plates are given in Table 1. After the given exposure period, all witness plates were aseptically placed into 50-mL polypropylene disposable sterile centrifuge tubes (Fisher Scientific Co., Georgia, Cat. # 05-539-6).

Collection of particles and fibers. To determine the extent of particulate contamination on the surfaces, a tape lift technique was performed {(Abrams and Hedgeland, 1989) per GSFC-TLS-PR-7324-01. Contamination control procedure for the tape lift sampling of surfaces.} Particulate materials were counted and distinguished individually according to its size using an optical microscope. Resultant data are an approximate diameter measurement for particles and a length measurement for fibers. All particulate materials larger than 500 μm in size were identified individually.

Microbial examination. Each witness plate retrieved from SAF was placed into 30 mL of sterile phosphate buffered rinse solution (To prepare stock buffered distilled water, dissolve 34.0 g of potassium dihydrogen phosphate (KH_2PO_4) in 500 mL of distilled water, adjust to pH 7.2 ±0.1 using one normal NaOH, dilute to one liter with distilled water. To prepare a working solution, add 1.25 mL of the stock buffer solution to one liter of distilled water.) and sonicated for 2 min. The rinse solution was aseptically pipetted out and split into 2 parts (15-mL each). One part of the rinse solution along with the witness plate were subjected to heat-shock (80°C for 15 min) and the other part was not heated. Two-mL aliquots were placed into sterile petri dishes in several replicates. The whole witness plate was also placed into a sterile petri dish. Molten Trypticase Soy Agar (TSA, Difco) was aseptically poured into these petri dishes containing samples and the contents were gently mixed to obtain uniform distribution of microbes. All plates were incubated in an inverted position at 32°C for 3 to 7 days and colony forming units (cfu) were counted and recorded.

Purification and maintenance of isolated microbes. All microorganisms, including fungi, yeasts and bacterial isolates were at least streaked thrice in TSA and checked for their purity before stored in a maintenance media. Aliquots (300 µL) of fully grown purified culture in Trypticase Soy Broth (TSB, Difco) was added into 30% sterile glycerol (600 µL) solution and frozen at –80°C until further characterization was performed. Step-wise freezing process (4°C, –4°C, –20°, –80°C) was undertaken to avoid any stress that would damage the viability of the microbes. All strains were stained for Gram-reaction (Fisher Scientific Gram stain set diagnostic kit, Catalog # SG 100D) and DAPI staining (Kepner et al. 1994 Use of fluorochromes for direct enumeration of total bacteria in environmental samples: past and present. Microbiol Rev. 58:603-615). The ability to grow at various temperatures and at sodium chloride concentrations was used to select strains. All the isolates that showed growth at 60°C were further characterized. However, some representatives of bacteria that were morphologically different but not growing at 60°C were also included.

Phenotypic characterization. Routine biochemical tests were carried out according to established procedures (Venkateswaran *et al.*, 1989, West & Colwell, 1984, Baumann *et al.*, 1984). The ability to grow at a NaCl concentration of 1 to 10% was determined in T_1N_1 liquid medium (Venkateswaran *et al.*, 1989), and the ability to grow without NaCl was determined in 1% sterile tryptone water. Temperature tolerance at 60°C was performed in TSB. The API CHB 50 kit was used for the Gram-positive spore-forming rods and the API STAPH kit was used for Gram-positive coccoid isoaltes (Biomerieux identification system). Identification of the test isolate was carried out by computing all test results into the Biomerieux database. For the determination of cell shape and size and the detection of flagella, cells were negatively stained with osmium chloride according to the methods of Cole and Popkin (1981) and observed with a Hitachi (H-600, Tokyo, Japan) transmission electron microscope.

Fatty acid analysis. Cells were cultivated overnight in liquid TSB medium at 30°C with vigorous shaking. Cellular fatty acids were extracted from lyophilized cells, methylated and analyzed by gas chromatography (Ringelberg *et al.*, 1994). Fatty acid methyl esters (FAME) were analyzed on a cross-linked 5% phenyl silicone capillary column (0.2 mm internal diameter; 25 m long; Hewlett Packard, Palo Alto, Cali.) with a HP 5890A gas chromatograph. The column was kept at an initial temperature of 80°C for 2 min and the temperature was programmed to increase from 80 to 150°C at 10°C/min, then to 282°C at 3°C/min and finally held at 282°C for 5 min. The temperature of the injector was maintained at 250°C and the detector at 290°C. The FAME peaks were identified by retention time comparisons against authentic fatty acid methyl ester standards and quantified by integration of peak areas. In addition, FAME peaks were also identified by mass spectrometry (electron impact at 70eV) including verification of double bond position via DMDS derivatization (Ringelberg *et al.*, 1994).

Molecular characterization. Purified genomic DNA (Johnson, 1981) from liquid-grown cultures was quantified and ~10 ng of DNA was used as the template for PCR amplification. PCR assays were performed in a GeneAmp PCR System 9700 Thermal Cycler (Perkin Elmer Corp., Foster City, Calif.).

(i) DNA-DNA hybridization. Cells were suspended in 0.1M EDTA solution (pH8.0) and digestion of cell wall was carried out by treating the cells with lysozyme (final concentration, 2mg/ml) for bacilli or labiase (final concentration, 2mg/ml) for cocci. The DNA was isolated by standard procedures (Sambrook et al., 1989). DNA-DNA hybridization was carried out as described previously (Satomi et al.,1997; Satomi M, Kimura B, Mizoi M, Sato T, and Fujii T. Int J Syst Bacteriol 1997 47:832-836. Tetragenococcus muriaticus sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce.).

(ii) 16S rRNA. Universal primers (Bact 11 and 1,492) were used to amplify the 1.4-kb PCR fragment as per the protocols established by Ruimy *et al.* (1994). Amplicons thus generated were sequenced directly following purification on Qiagen columns (Qiagen, Valencia, Cali.).

(iv) Sequencing. The identity of a given PCR product was verified by sequencing using the dideoxy chain termination method with Sequenase DNA sequencing kit (United States Biochemical Corporation, Cleveland, Ohio) and with an ABI 373A automatic sequencer as recommended by the manufacturer (Perkin-Elmer Applied Biosystems, Foster City, Calif.).

Phylogenetic analysis and sequence alignment. The phylogenetic relationships of organisms covered in this study were determined by comparison of individual 16S rRNA sequences with other sequences already exist in the public database. Evolutionary trees were constructed with the PAUP (Swofford, 1990), PHYLIP (Felsenstein, 1990) and ARB program packages (Strunk and Ludwig, 1995). Nucleotide sequence accession numbers are given in Table 1.

RESULTS

Collection of witness plates. Table 1 details the retrieval history of various witness plates deployed in SAF. About 20 to 25 replicates of 5 different types of witness plates were removed and individually placed into 50-mL screw-capped sterile centrifuge tubes. Although we deployed these witness plates on various dates, we have retrieved the samples on the same day. The exposure time varies from 232 days to 278 days.

Distribution of particulate materials and fibers. Table 2 shows the distribution of particulate materials and fibers collected on various witness plates during the present study. In general, particles of the size 5 to 150 μm were collected on all types of witness plates. A clear pattern of particle distribution was noticed in the spacecraft qualified paint coated witness plates. The smaller size particles were more abundant in the paint coated witness plates and the profusion decreased with increased particles size. In contrast, pure stainless steel witness plates accumulated more mid-range size (21-100 μm) particles and the abundance of particles decreased when the particles sizes decreased. In terms of fibers, the NS43G paint coated surface attracted more fibers followed by stainless steel, S13GLO-1, Z307 and 463-3-8 paint coated surfaces.

Microbial examination. Heat-resistant and vegetative microbial population enumerated from various witness plates that were exposed at SAF are tabulated (Table 3). In general, the paint-coated witness plates attracted more microbial population than the pure stainless steel plates. Such microbial abundance is well correlated with abundance of particulate materials trapped on the witness plates. In other words, NS43G-coated witness plates had trapped more particles and number of microorganisms isolated was also high when compared to other witness plates. However, the fibers counted on various witness plates showed no relationship with the microbial counts. In general, both heat-resistant (3 to 1.5×10^1 cfu/cm^2) and vegetative (5 to 6.9×10^1 cfu/cm^2) microbial contamination transferred through particulate materials is not heavy and it is minimal in terms of microbial load. In addition to the bacterial population, spatial distribution of yeast and fungal population were noticed in these witness plates and were insignificant. Further characteristics of microbes other than bacteria were not carried out during this study.

Isolation of microbial population. Bacterial colonies of various morphotypes were carefully selected with an emphasis on representing all witness plates. A total of 89 isolates were picked and purified before storing them in glycerol stocks. Thirty-nine and 50 isolates were picked from the samples that were treated with 80°C for 15-min and unheated samples, respectively. About 44% of 39 heat-tolerant (80°C for 15-min) isolates and 5 of the 50 strains isolated from samples that were not heat-shocked showed growth at 60°C. Similarly, about 50% of these 89 isolates showed very good growth at 10% NaCl concentration. All isolates were able to ferment glucose. Only 5% of these isolates were coccoid and others were rod shaped. The purified bacterial isolates were stained for Gram-reaction and all were stained as Gram-positive. Morphology was checked by DAPI and BacLight (Molecular Probes Inc.) staining. The positioning of spores were checked after staining with malachite green.

Phenotypic characterization. Representatives of rod-shaped (22 strains; API CHB50 test strip) and coccoid-shaped (5 strains; API STAPH test strip) bacterial cultures were tested for their physiological characteristics features. All coccoid and one rod-shaped isolates (FO-092) were picked for their morphological novelty and other isolates were selected for their profound growth at higher temperature (60°C). The results of various biochemical reactions recorded for rods (Table 4) and coccoid (Table 5) isolates are depicted. Based on the Biomerieux database only 9 rods and 1 coccoid-shaped bacteria were identified to its species level. All rod-shaped bacteria were presumptively identified as *Bacillus* sp. and fell into two groups *B. licheniformis* and *B. pumilus.* The coccoid-shaped isolates were identified by phenotypes as the members of the genera *Micrococcus* or *Staphylococcus.*

Fatty acid analysis. The FAME compositions of various strains isolated are shown in Table 6. The FAME profiles display only those fatty acids comprising 0.1% of the total or greater. The elimination of minor fatty acids from consideration had no effect on any of the relationships described here. All of the values presented here were recalculated to show the relative abundance of the various fatty acids. Terminally branched saturated FAME (58.3 to 99.2%) peaks were the major lipids when compared to straight chain saturated FAME (2.9 to 41.7%) and monounsaturated FAME (0.8 to 30.2%) lipid classes.

Among coccoid-shaped groups, members of *Micrococcus* genus (FO-017a, FO-084a) had shown similar FAME pattern as that of *Bacillus* sp. *M. luteus* FO-017a secreted predominantly 15:0 anteiso (80% of its total fatty acid composition). However, strain FO-074a (19% monounsaturated) and *S. epidermidis* FO-036a (41% straight chain saturated) showed different patterns in their FAME composition. A distinct fatty acid signature (20:0; 23.4%) was noticed in *S. epidermidis* FO-036a followed by 11% incidence of 18:0.

C15:0 anteiso was predominant in *Bacillus* sp. Among various groups of *Bacillus, B. licheniformis*-group yielded significant amount of 15:0 anteiso (33 to 38%), 15:0 iso (20 to 35%) and 17:0 anteiso (11 to 16%). One other unidentified *Bacillus* sp. FO-092 also fell into this category. *B. pumilus*-group (FO-033, FO-036b, FO-038) generated 15:0 iso (45 to 49%) in significant quantity than 15:0 anteiso (25%). *B. cereus*-group (FO-011, FO-080) produced monounsaturated acids to the range of 20 to 30% of their total FAME composition. The strains FO-003 and FO-029a were high in terms of C15:0 anteiso (40 to 41%) followed by C15:0 iso (13 to 20%). Unlike seen in any other group, *B. cereus*-group produced significant amounts of monosaturates (20 to 30%) but terminally branched saturates were more abundant (60 to 70%) than straight chain saturates (8 to 9%). The mucoid variety of *B. cereus*-group strain FO-080 synthesized more iso 17:1 w10c (12%) and the other member of this group strain FO-011 produced abundant 16:1w7c (10%). It is noteworthy to state that all *B. liccheniformis* strains did not show similar FAME profiles although all these strains cultured under same conditions.

Molecular phylogenetic analysis.

16S rRNA sequence analysis. The 1.4-kb nucleotide sequences of the 16S rRNA covering base positions 11 to 1492 (*E. coli* numbering), were determined for 23 isolates. Among these isolates, 4 strains of coccoid-shaped and 19 strains of rod-shaped isolates were selected for sequencing 16S rRNA fragment. The 16S rRNA sequences of all 23 strains were aligned and a neighbor joining phylogenetic tree constructed (Fig. 1).

There were 3 different types observed among coccoid-shaped bacteria. They were the members of the genera *Micrococcus, Staphylococcus* and *Planococcus.* Two isolates that have high similarities with *Micrococcus* did exhibit >99% and 98% similarities with *M. luteus* and *M. lylae*, respectively. The phylogenetic tree based on the 16S rRNA analysis indicates that the *Micrococcus* species form a group independent of the others. *Staphylococcus* and *Planococcus* species form distinct independent groups and these 2 groups were clustered in the main stalk of *Bacillus* species. The strain FO-036a showed 99.8% similarities with both *S. epidermidis* and *S. capitis*. However, one strain FO-074a had low similarities with any of the sequence available in the public database. The genus *Planococcus* is the closest relative to the strain FO-074a and shown about 96.9% similarities with *P. citreus*. The strain FO-074a grouped among *Planococcus* members in the phylogenetic tree forms a deep distinct branch.

Variation in 16S rRNA sequences among rod-shaped strains revealed 4 groups. These are related to *B. cereus* (2 strains), *B. pumilus* (3 strains), *B. licheniformis* (13 strains), and *B. circulans* (1 strains) groups. This grouping was drawn on the basis of their proximity to respective type strains. A group of 13 strains that are related to *B. licheniformis*, while somewhat heterogeneous in 16S rRNA sequence (97.2 to 99.8% similarities) appeared to form a cluster. Among the *Bacillus* group, the phylogenetic tree also exhibited 4 distinct clades. Although the strain FO-003 was identified as *B. subtilis* by FAME analysis, the phylogeny tree grouped this strain among *B. licheniformis*-group. Both *B. licheniformis* and *B. pumilus* groups occupy the same stem in the phylogenetic tree where the strain FO-029a that showed higher similarities with *B. subtilis* (99%) falls into the *B. pumilus*-group. The strain FO-011 that was identified as *B. cereus* exhibited 99.1% similarities with both *B. cereus* and *B. thuringiensis*. Likewise, the other member of this cluster the strain FO-080 that exhibited mucoid nature in its morphology did cluster with *B. mycoides* (99.2%), although this strain showed a 98.9% similarities with both *B. cereus* and *B. thuringiensis*. The lone member of the fourth group the strain FO-092 that have high similarities with *B. circulans* formed a cluster with *B. benzeovorans* (98.1%) and *B. circulans* (98.7%). Although the strain FO-092 exhibited high similarities with *B. firmus* (98.3%), *B. megaterium* (97.6%), and *B. cohnii* (97.1%), these 3 species bifurcated from FO-092-cluster at the stem of the tree.

DNA-DNA hybridization. DNA-DNA hybridization was performed to examine relatedness between the species isolated from SAF and representatives of the genera *Bacillus, Micrococcus, Staphylococcus* and *Planococcus*. The percentage similarities of *Bacillus* strains examined are given in Table 7 and for coccoid-shaped strains in Table 8. As per the recommendations of Stackebrandt and Goebel, (1994), the identity of the bacterial species was determined as the same when the DNA-DNA reassocciation values between the type species and test strain were above 70%.

Among 19 *Bacillus* strains tested, the DNA reassociation percentage range for 10 strains was 77 to 100%, with *B. licheniformis*. Three strains were closest to *B. pumilus* (65 to 85%). One of the two strains identified as *B. cereus*-group (FO-080) showed 77% similarities with *B. mycoides* where as the non-mucoid strain FO-011 exhibited 63% similarities with *B. cereus*. Two strains (FO-032 and FO-035b) that shows higher 16S rRNA sequence similarities (99%) with *B. licheniformis* did not exhibit higher DNA reassociation values (19% and 38%). Similarly, the strain FO-029a that shows 99% 16S rRNA similarities with *B. popilliae* did exhibit 77% similarities with *B. subtilis* type strain. One morphologically novel strain FO-092 that produces extremely big spores (data not shown) showed no relationship with any of the *Bacillus* species tested. Furthermore, FO-092 strain that exhibited 98.7% 16S rRNA sequence similarities showed only 21% DNA reassociation values with *B. circulans*.

DISCUSSION

Prokaryotes are believed to have inhabited Earth for more than 3.5 billion years and yet have remained simple and small throughout their evolutionary history. Their diversity is expressed in terms of physiology and metabolism. Prokaryotes have optimized their biochemistry for the uptake and utilization of a wide variety of nutrients thereby creating unique pathways and genetic regulation to meet a variety of conditions within nature. Deep in the subsurface of the earth, new species are being identified that survive for long periods of time without growing or being metabolically active. The marine deep-sea muds and thermal vents as well as hot springs and geysers appeared to be favorite sampling sites today. A spacecraft building facility for a life detection mission is often guarded by stringent quality control measures. Because of controlled air circulation, desiccation, moderately high temperature, and low-nutrient conditions, the atmosphere of SAF should be considered as extreme environment and microbes might find difficult to thrive under such conditions. Evidently, many bacterial strains isolated from the SAF exhibited intense growth at 60°C (44%), and 10% NaCl (50%). This substantiated the fact the SAF is an extreme environment. Majority of these thermotolerant and halotolerant isolates were identified as *Bacillus* species.

The ability to determine the thermal resistance of naturally occurring airborne bacterial spores associated with spacecraft and their assembly areas has been hindered by lack of an effective collecting system. Efforts to collect and concentrate spores with air samplers or from air filters have not been successful (Puleo et al. 1975). A fallout method was developed where sterile Teflon ribbons were exposed in pertinent spacecraft assembly areas and subsequently analyzed for microbial population that survives with dry heat (125 and 113°C; Puleo et al. 1975). Likewise, naturally occurring airborne bacterial spores were collected on Teflon ribbons in selected spacecraft assembly areas where Viking spacecraft was built and subjected to thermal inactivation experiments (105 to 135°C). Majority of heat survivors recovered at these temperatures was phenotypically identified as *Bacillus* species.

Microbiological assessment of two Viking spacecraft during assembly and testing at Cape Canaveral and the Kennedy Space Center was reported. Levels of bacterial spores per square meter on the Viking Lander Capsules were 9.7×10^1 to 1.6×10^2 prior to dry-heat sterilization. The ranges of aerobic mesophilic microorganisms detected on the Orbiters at various sampling events were 2.3×10^2 to $8.9 \times 10^3/m^2$. About 75% of 1,300 isolates were microorganisms considered indigenous to humans (*Staphylococcus* or *Micrococcus*-groups); the remaining isolates were associated with soil and dust (*Bacillus*-group) in the environment (Puleo et al. 1977). Similar results were obtained with previous automated spacecraft but slightly lower than those observed for manned (Apollo) spacecraft (Puleo et al. 1973). Our results confirmed that the JPL SAF High Bay-1 exhibited lower microbial burden and their taxonomical characterization revealed a similar microbial profile as that of Viking mission. In other words, the Precursor spacecraft that used to verify spacecraft level flight article assembly and test operating procedures at the launching site had high incidence of the members of the family *Bacillaceae*. The witness plates employed in this study mimics the Precursor spacecraft in their microbial profile rather than the Viking 1 and 2 spacecraft.

Until recently, bacterial taxonomists have had little option but to rely on patterns of physiological traits to classify their subjects. Colony appearance, characteristic smells, pigmentation and other attributes of organisms in culture can be of dubious value in terms of useful taxonomic insight and can potentially lead to the assignment of misguided associations between loosely affiliated or unrelated organisms. While it is possible to systematize any group of isolates on the basis of phenotypic characters, the comparative value of a given trait or group of traits over another remains difficult to assess. This is not meant to infer that phenotypic considerations have lost standing in the determination of taxonomic relationships. Indeed, the Ad Hoc Committee on the Reconciliation of Approaches to Bacterial Systematics concluded that phenotypic consistency must remain the bottom line in polyphasic taxonomy (Wayne *et al.*, 1987). Rather, the limitations of classical phenotypic taxonomy argue for the inclusion of independent measures for the validation of bacterial relationships.

It is possible to differentiate all *Bacillus* species based on physiological traits, the required biochemical testing is labor intensive, costly and potentially prone to experimental error also. Molecular methods are tacitly less susceptible to artifactual misinterpretation than culture-based approaches. With the advent of these techniques, a well-defined series of criterion for the designation of taxa now exist that function independently of sometimes phylogenetically arbitrary physiological and morphological boundaries. The basic unit of taxonomy is the species, which is defined as a group of strains, including the type strain, sharing 70% or greater relatedness over the entire genome (Wayne *et al.*, 1987). An extensive review of the literature by Stackebrandt and Goebel (1994) revealed that organisms with less than 97% similarity over the 16S rRNA gene do not yield DNA reassociation values of more than 60% hence, rRNA sequence analysis may be used as a surrogate for DNA reassociation. On the other hand, rRNA similarity of greater than 97% does not necessarily indicate that any two isolates is of the same species. For example, although *V. parahaemolyticus* and *V. alginolyticus* show very high rRNA similarity (99.9%; Ruimy *et al.*, 1994), phenotypically they are easily distinguished and regarded as distinct species (Venkateswaran *et al.*, 1989). Similarly, *B. cereus, B. thuringiensis,* and *B. mycoides* cannot be differentiated at the 16S rRNA level though *B. thuringiensis* can be physiologically distinguished by virtue of its insecticidal crystal protein (Turnbull and Kramer, 1991). For these reasons, any taxonomic scheme must show phenotypic as well as molecular consistency (Wayne *et al.*, 1987).

Based on phenetic, and chemotaxonomic data and phylogenetic information, the genus *Bacillus* went through various changes. Among 117 recognized *Bacillus* species, 41 were reclassified into seven new genera. They are: *Alicyclobacillus* (Wisotzkey et al., 1992), *Aneurinibacillus* (Shida et al., 1996), *Brevibacillus* (Shida et al., 1996), *Gracilibacillus* (Waino et al., 1999), *Paenibacillus* (Shida et al., 1997), *Salibacillus* (Waino et al., 1999), and *Virgibacillus* (Heyndrickx et al., 1999). In accordance with the consensus molecular definition of the species, it has been reported the subset of strains containing 97% or greater similarity to the type strain as legitimate species (Wayne *et al.*, 1987). However, members of the genus *Bacillus*, a rather heterogeneous group, possessed 16S rRNA sequence similarities as low as 94% or as high as 99.9% between phenotypically distinct species. For example, *B. licheniformis* showed low and high similarities with *B. agaradherens* (93%), and *B. lautus* (99.7%), respectively. Furthermore, recently *B. lautus* was reclassified as *Paenibacillus* sp. (Heyndrickx et al., 1996) based on its low DNA-DNA hybridization values.

Comparative phylogeny of 16S rRNA in the family *Bacillaceae* was recently documented (Achoauak et al., 1999). The sequence analysis has demonstrated that the genus *Bacillus* consists of at least five monophyletic clusters (Ash et al., 1991). *Bacillus* species that produce acid from variety of sugars including glucose were classified under rRNA group 1. Most of these species were able to grow at least weakly in absence of oxygen. Spores of these species were ellipsoidal and did not swell the mother cell. These species are considered the "subtilis group" because of their otherwise similar physiological properties (Priest, 1993). All Gram-positive rods isolated from JPL SAF High Bay-1 were fall into the rRNA group 1. The members of the "subtilis group" (*B. cereus, B. licheniformis, B. pumilus,* and *B. subtilis*) are prevalent in soils, dusts, and marine and freshwater habitats. However, 3 of the 19 strains examined did not match with any of the established *Bacillus* species and need further characterization. This microbial profile of JPL SAF High Bay 1 resembles the Kennedy Space Center SAF, Florida where Precursor spacecraft of Viking mission was kept. However, the human activity that occurred during assembly of Viking 1 and 2 spacecraft shed more human-associated microbes (*Micrococcus-Staphylococcus* group) than members of the family *Bacillaceae*.

Because 16S rRNA sequence analysis have proven inadequate for the differentiation of *Bacillus* species, DNA-DNA hybridization was used to verify our assertion that any of these strains described here deserves species status. The conventional biochemical tests, FAME analysis and 16S rRNA sequence results did not articulate in defining the species status of the given strain during this study. Only 4 out of 19 *Bacillus* strains and one of the five coccoid-shaped shaped strains were identified as the same species by all four methodologies. It is clear from results that the biochemical test profile did not differentiate the Gram-positive bacteria at their species. This might be due to the fact that the microbes isolated from SAF would have forced to shed some of their genetic characters to adopt themselves to the extreme environmental conditions thereby changing their phenotypes. The results of FAME and 16S rRNA profiles (85%), and 16S rRNA and DNA-DNA hybridization profiles (75%) were in accordance in identifying as the same species. DNA hybridization studies revealed existence of 4 different *Bacillus* species among 19 strains tested and further supported to describe 3 new *Bacillus* species (data not shown). Although the strains FO-032 and FO-35b showed higher similarities (>98%) with *B. licheniformis* by 16S rRNA sequence analysis and phenotypically related, DNA-DNA reassociation values were only 19 and 38%, respectively. It is interesting to note that the strain FO-092 had a clear match with *B. circulans* by phenotypic data, FAME profiles, and 16S rRNA sequence similarities (98.7%). However, DNA hybridization study (21% with *B. circulans*) clearly indicates that the strain FO-092 should be regarded as distinct.

The work at hand represents a comprehensive effort to impose a logical phylogenetic framework on the growing collection of SAF isolates. While it appears likely that additional isolates representing novel species will be discovered in the future, the species considered here already indicate that SAF contains formerly unappreciated diversity. This study illustrates the utility of molecular and chemotaxonomical approaches for determining phylogenetic relationships in SAF. It is possible to build upon the taxonomic foundations established by the polyphasic approach to design gene probes for the rapid and efficient screening of microbes to ensure the microbial quality of the facility.

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Table 1. Nature, placement position, and exposure time of various witness plates deployed in a spacecraft assembly facility during this study.

Nature of the witness plate	Nature of the paint	Location	Date & Time Installed	Date & Time Retrieved	# of witness plates Retrieved	Exposure, days (months)
Stainless steel	-	North	6/11/98 3 p.m.	3/16/99 2:00 p.m.	20	278 (9.3)
NS43G-painted	off-white conductive paint (silicate binder)	West	7/27/98 11 a.m.	3/16/99 2:00 p.m.	25	232 (7.7)
S-13GP/LO-1-painted	non-conductive white silicone elastomer based	West	7/9/98 9:30 a.m.	3/16/99 2:00 p.m.	25	250 (8.3)
463-3-8-painted	non-conductive black epoxy	West	6/26/98 3:30 p.m.	3/16/99 2:00 p.m.	20	263 (8.8)
Z307-painted	electrically conductive flat black epoxy	West	7/27/98 11 a.m.	3/16/99 2:00 p.m.	25	232 (7.7)

Table 2. Particlulate matter associated with various witness plates exposed at SAF facility

Size range of the particles isolated or fibers	Number of particles or fiber size isolated from the witness plates of:				
	NS43G	Stainless steel	S13GLO-1	Z307	463-3-8
Particles of the size range of:					
5-10µ	3180	N/A	1260	1090	978
11-25µ	352	64	336	87	93
26-50µ	131	181	163	68	74
51-100µ	12	210	61	31	22
101-150µ	3	11	7	0	0
151-250µ	0	0	1	3	0
251-350µ	0	0	0	0	0
351-500µ	0	0	0	0	0
>500µ	0	0	0	0	1
Fibers:	10µ X 1100µ	10µ X 1680µ	10µ X 400µ	10µ X 900µ	10µ X 310µ
	10µ X 1600µ	10µ X 1680µ	10µ X 440µ	15µ X 240µ	20µ X 500µ
	10µ X 2000µ	15µ X 4500µ	10µ X 80µ	20µ X 200µ	30µ x 200µ
	10µ X 2400µ	15µ X 4500µ	15µ X 120µ	20µ X 80µ	30µ x 5000
	10µ X 2400µ	15µ X 4800µ	15µ X 120µ	20µ X 920µ	40µ X 250µ
	10µ X 280µ	15µ X 4800µ	15µ X 180µ	30µ X 280µ	
	10µ X 3200µ	20µ X 1400µ	15µ X 300µ	40µ X 400µ	
	10µ X 400µ	20µ X 1400µ	15µ X 440µ	40µ X 500µ	
	10µ X 500µ	20µ X 1600µ	15µ X 600µ		
	10µ X 800µ	20µ X 2400µ	15µ X 800µ		
	10µ X 900µ	20µ X 360µ	15µ X 900µ		
	15µ X 120µ	20µ X 360µ	20µ X 100µ		
	15µ X 1400µ	20µ X 500µ	20µ X 1200µ		
	15µ X 1500µ	20µ X 500µ	20µ X 1300µ		
	15µ X 1600µ	20µ X 5000µ	20µ X 300µ		
	15µ X 1600µ	20µ X 5000µ	20µ X 750µ		
	15µ X 2200µ	20µ X 800µ	20µ X 760µ		
	15µ X 2400µ	20µ X 800µ	20µ X 800µ		
	15µ X 2800µ	20µ X 900µ	25µ X 300µ		
	15µ X 2800µ	20µ X 900µ	30µ X 500µ		
	15µ X 3200µ	30µ X 400µ	30µ X 500µ		
	15µ X 350µ	30µ X 400µ	6µ X 600µ		
	15µ X 400µ	30µ X 500µ			
	15µ X 400µ	30µ X 500µ			
	15µ X 650µ	30µ X 800µ			
	15µ X 680µ	30µ X 800µ			
	15µ X 930µ	30µ X 900µ			
	15µX 400µ	30µ X 900µ			
	20µ X 80µ	40µ X 800µ			
	20µ X 250µ	40µ X 800µ			
	20µ X 400µ				
	20µ X 500µ				
	20µ X 1000µ				
	20µ X 1200µ				
	20µ X 1800µ				
	20µ X 2400µ				
	20µ X 2400µ				
	20µ X 700µ				
	25µ X 2800µ				
	25µ X 3200µ				
	30µ X 400µ				

Table 3. Microbial population isolated from various witness plates exposed in spacecraft assembly facility

Treatment	Number of bacteria (cfu/cm^2) isolated from the witness plates of:				
	Stainless steel	Z307	S13GLO-1	NS43G	463-3-8
After heat-shock treatment	6	10	3	14.5	1.5
Before heat-shock treatment	5	16	36.5	68.5	30.5

Table 4. Biochemical characteristics of Gram-positive rods isolated from space craft assembly facility

Test	Characteristic reaction in the strain of:																					
	FO-003	FO-011	FO-017b	FO-022a	FO-024	FO-026	FO-028	FO-029a	FO-030	FO-031	FO-032	FO-033	FO-035b	FO-036b	FO-037	FO-038	FO-054	FO-074b	FO-080	FO-084b	FO-085	FO-092
Enzyme production:																						
β-galactosidase	+	-	-	+	+	+	+	-	+	+	+	-	-	-	+	-	+	+	-	+	+	+
Arginine dihydrolase	+	+	+	-	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	+	-	-
Cytochrome oxidase	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-
Acetoin production	-	-	-	-	-	+	-	-	-	-	+	-	-	+	-	+	+	-	+	+	-	-
Gelatin liquefication	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-
NO_3 to NO_2	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-
NO_2 to Nitrogen gas	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Utilization of:																						
Glucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Mannitol	-	-	-	+	-	-	+	-	-	-	+	-	+	-	+	-	-	+	-	+	-	-
Inositol	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	-	-	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
Amygdalin	-	-	-	-	-	+	-	-	+	-	+	+	-	+	+	+	-	-	-	-	-	-
Fermentation of:																						
Glycerol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
L-Arabinose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Ribose	+	+	-	+	+	+	+	+	-	+	-	+	+	+	-	+	+	-	+	-	-	-
D-Xylose	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+
Adonitol	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Galactose	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	-	-	-	-	+
D-Glucose	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Fructose	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
D-Mannose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Inositol	+	-	-	+	+	+	+	+	-	+	+	+	-	+	-	+	+	-	+	+	-	+
Mannitol	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Sorbitol	+	-	-	+	+	+	+	+	+	+	+	-	+	-	+	-	+	+	-	+	-	+
α Methyl-D-mannoside	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-

Table 5. Biochemical characteristics of Gram-positive cocci strains* isolated from space craft assembly facility

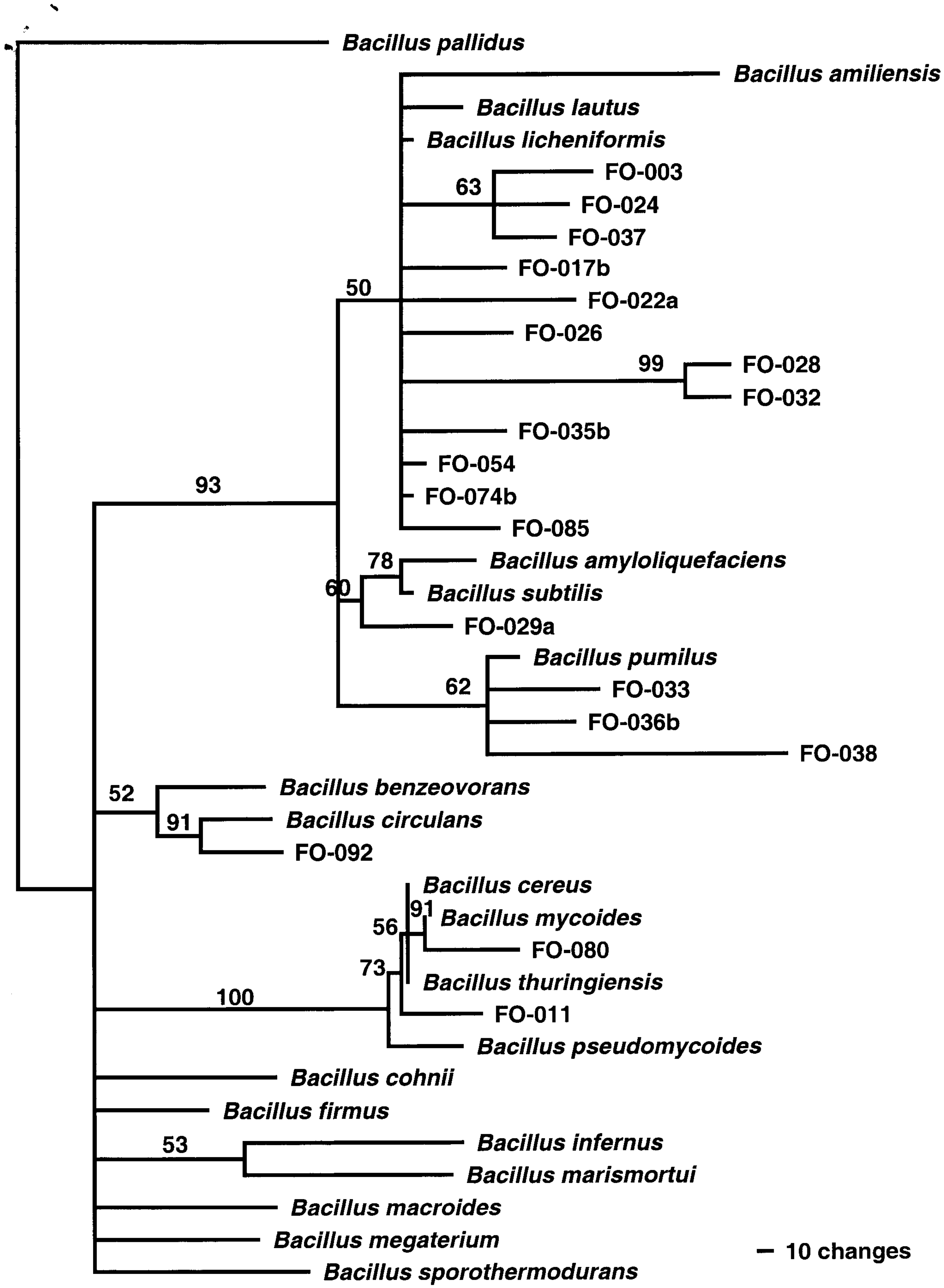
Test†	Characteristic reaction in the strain of:		
	FO-017a	FO-036a	FO-074a
Enzyme production:			
Alkaline phosphatase	+	+	+
Urease	-	+	-
Acetoin production	-	+	-
NO3 to NO2	-	+	+
Utilization of:			
D-Glucose	-	+	+
D-Fructose	-	+	+
D-Mannose	-	+	-
Maltose	-	+	-
Lactose	-	+	-
Sucrose	-	+	-

*Strains FO-061 and FO-084a did not show positive reaction in any of the API STAPH tests.

†None of the strains produced arginine dihydrolase. All strains fail to utilize D-trehalose, D-mannitol, xylitol, D-melibiose, raffinose, xylose, a-methyl-D-glucoside, and N-acetyl-glucosamine.

TABLE 7. DNA-DNA hybridization of various *microbial* species isolated from Spacecraft Assembly facility

Bacteria	Strain no.	Percentage similarities to labelled DNA from:															
		B. licheniformis ATCC 14580	*B. cereus* ATCC 49005	*B. circurans* ATCC 4513	*B. mycoides* ATCC 49005	*B. pumilus* ATCC 7061	*Bacillus* sp. FO-011	*Bacillus* sp. FO-029a	*Bacillus* sp. FO-036b	*Bacillus* sp. FO-085	*Bacillus* sp. FO-092	*S. epidermidis* ATCC	*S. capitis* ss. *capitis* ATCC 27840	*S. capitis* ss. *ureolyticus*ATCC 49326	*Planococcus citreus* ATCC	*Staphylocccus* sp. FO-036a	*Planococccus* sp. FO-074a
Bacillus sp.	FO-003	100						20		120							
Bacillus sp.	FO-011	14	54	9	46	6	100	9	3		17						
Bacillus sp.	FO-017b	86						12		109							
Bacillus sp.	FO-022a	95						12		99							
Bacillus sp.	FO-024	86						8		120							
Bacillus sp.	FO-026	87						12		110							
Bacillus sp.	FO-028	85						13		88							
Bacillus sp.	FO-029a	11	17	7	12	10	13	100	10	5	14						
Bacillus sp.	FO-032	19	15	4	12	8	13	14	8	6	12						
Bacillus sp.	FO-033	22	27	8	24	85	20	21	100		19						
Bacillus sp.	FO-035b	38	14	2	18	6	12	14	6	33	12						
Bacillus sp.	FO-036b	12	15	5	12	65	10	16	100		13						
Bacillus sp.	FO-037	114						16		110							
Bacillus sp.	FO-038	5				70			100								
Bacillus sp.	FO-054	83						9		88							
Bacillus sp.	FO-080	3	43	1	77	1	35	9	2		11						
Bacillus sp.	FO-084b	85						13		85							
Bacillus sp.	FO-085	77						11		100							
Bacillus sp.	FO-092	9	22	5	20	1	10	13	4		100						
B. benzoevorans	ATCC 49005		11	5	7	2	7	15	2		9						
B. cereus	ATCC 49005		100	5	45	1	63	6	1		4						
B. circurans	ATCC 4513		17	100	15	4	15	12	2		21						
B. cohnii	ATCC 51227		18	15	18	14	14	23	10		19						
B. firmus	ATCC 14575		21	9	16	15	15	23	11		19						
B. licheniformis	ATCC 14580	100						12		92							
B. mycoides	ATCC 49005		52	5	100	1	52	9	6		17						
B. pumilus	ATCC 7061		19	7	22	100	30	21	68		28						
B. subtilis	ATCC 49005		12	2	9	4	5	77	5		12						
B. thuringiensis	ATCC 49005		47	6	34	1	31	6	1		13						
S. epidermidis	ATCC															23	
S. capitis ss. *capitis*	ATCC 27840															75	
S. capitis ss. *ureolyticus*	ATCC 49326															74	
Planococcus citreus	ATCC																90
Staphylococcus sp.	FO-036a																
Planococcus sp.	FO-074a																


Bacillus pallidus
Bacillus amiliensis
Bacillus lautus
Bacillus licheniformis
63
FO-003
FO-024
FO-037
FO-017b
50
FO-022a
FO-026
99
FO-028
FO-032
FO-035b
FO-054
FO-074b
FO-085
93
78
Bacillus amyloliquefaciens
60
Bacillus subtilis
FO-029a
62
Bacillus pumilus
FO-033
FO-036b
FO-038
52
Bacillus benzeovorans
91
Bacillus circulans
FO-092
Bacillus cereus
91
Bacillus mycoides
56
FO-080
73
Bacillus thuringiensis
100
FO-011
Bacillus pseudomycoides
Bacillus cohnii
Bacillus firmus
53
Bacillus infernus
Bacillus marismortui
Bacillus macroides
Bacillus megaterium
10 changes
Bacillus sporothermodurans

Test	FO-003	FO-011	FO-017b	FO-022a	FO-024	FO-026	FO-028	FO-029a	FO-030	FO-031	FO-032	FO-033	FO-035b	FO-036b	FO-037	FO-038	FO-054	FO-074b	FO-080	FO-084b	FO-085	FO-092
	Characteristic reaction in the strain of:																					
α Methyl-D-glucoside	+	-	-	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	-	+	+	+
N Acetyl glucosamine	+	+	-	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Amygdalin	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Arbutin	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Esculin	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salicin	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cellobiose	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Lactose	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+
Melibiose	-	-	+	-	-	-	-	+	-	-	-	+	-	+	-	-	-	-	-	-	-	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
Trehalose	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Inulin	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Melezitose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Raffinose	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+
Starch	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	+
Glycogen	-	-	-	-	+	-	+	+	-	+	-	-	-	-	-	-	+	-	+	-	-	-
Xylitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gentiobiose	-	-	-	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-	-	-	-	+
D-Turanose	-	-	+	-	-	+	-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	+
D-Lyxose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
D-Tagatose	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	-	+	+	+
L-Fucose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
D-Arabitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+
Gluconate	+	-	-	+	-	+	+	-	-	-	+	-	+	-	+	-	+	-	-	+	-	+
2-keto-gluconate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

None of the strains produce lysine or ornithine decarboxylases, urease, tryptophane deaminase, hydrogen sulfide, and indole. All strains fail to utilize citrate, sorbitol, rhamnose, melibiose and arabinose. None of the strains ferment erythritol, L-xylose, β Methyl-xyloside, L-sorbose, dulcitol, D-fucose, L-arabitol, and 5-ketogluconate.

Table 6. Fatty acid methyl ester composition of various bacterial strains isolated from spacecraft assembly facility

Fatty acid	Percentage of fatty acid methyl esters produced in the bacterial strains:																			
	FO-003	FO-011	FO-017A	FO-017B	FO-022A	FO-024	FO-028	FO-029A	FO-032	FO-033	FO-036A	FO-036B	FO-038	FO-054	FO-074A	FO-074B	FO-080	FO-084A	FO-084B	FO-085	FO-092
11:0 anteiso																		1.3			
12:0 iso		1.2															1.4				
13:0 iso		7.9								0.5	1.2	0.5	0.4				7.6	1.2			2.9
13:0 anteiso		2.0	1.9														1.9	4.4			
14:0 iso	1.0	7.1	2.4		0.8	0.8	0.7	2.3	1.0	2.1	5.5	1.7	1.5	0.6	9.1	0.8	5.0	2.5	1.0		6.6
14:1 w5c																		1.1			
14:0		2.9			0.5	0.5	0.5	1.1	0.8	1.9	2.2	1.8	1.5	0.4		0.4	3.0	3.7	0.5		12.3
15:0 iso	19.7	26.1	10.6	28.4	28.7	30.3	33.6	13.2	25.4	45.6	13.8	47.0	49.0	28.1	7.2	31.5	20.9	23.2	34.1	33.2	26.4
15:0 2OH			0.8																		
15:0 anteiso	41.2	7.3	80.2	38.2	35.5	34.8	34.1	40.0	32.8	25.6	26.6	25.6	25.5	36.6	39.9	33.7	4.5	57.5	32.6	36.7	32.2
15:0															4.4						
16:1 w7c alcohol		2.0			0.8	0.7	0.9			1.3		1.3	1.1	1.1	11.3	1.2	4.6		1.2		1.8
16:0 iso	4.8	9.6	2.1	3.1	3.7	3.3	2.6	4.6	4.0	3.4	1.9	3.0	2.7	2.3	9.0	2.7	10.2		2.7	3.9	2.1
16:1 w9c																					
16:1 w11c	2.1				1.1	1.1	1.5	4.8	0.9	3.2		3.2	2.9	2.1	2.8	1.9	4.0		1.6		7.5
16:0	5.3	5.3		2.9	3.3	3.1	2.8	10.2	6.1	4.0	4.7	3.4	3.2	2.5	1.4	2.6	6.2		2.5	3.6	5.2
iso 17:1 w5c		2.8																			
iso 17:1 w10c	1.4	3.6		2.0	1.9	1.9	2.4	1.6	0.7	2.1		2.3	2.1	2.6	0.9	2.8	11.8		3.1		
17:1 w8c																					
17:1 anteiso A		1.1																			
17:0 iso	6.3	6.5		8.2	8.1	8.4	7.4	7.8	11.5	5.3	3.3	5.1	5.3	6.5	1.8	7.6	6.9		7.4	7.8	
17:0 anteiso	16.1	1.6	2.1	15.3	14.1	13.6	11.4	12.2	15.6	4.3	2.8	4.4	4.3	14.2	4.7	12.2	2.2		11.0	14.8	3.0
17:0															2.5						
18:1 w9c															0.9						
18:0								1.0	1.1		11.0										
18:0 iso											0.6				1.2						
19:0 iso											2.1										
19:0 anteiso											0.7										
19:0											0.4										
20:0											23.4										
Sum of 13:0 3OH/15:1 i I/H																		2.6			
Sum of 14:0 3OH/16:1 iso I		3.2																			
Sum of 15:0 iso 2OH/16:1w7c		9.8															7.1	2.4			
Sum of 17:1 anteiso B/iso I	2.0			1.9	1.6	1.5	2.0	1.2		0.8		0.8	0.7	2.9	3.1	2.5	2.7		2.5		